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The health effects of fetal microchimerism can be modeled in companion dogs

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Fetal microchimerism (FMC) has been described to have a range of effects on health and disease. Y-chromosomal DNA has been detected in Golden Retrievers suggesting persistent FMC. In that report, nine dogs had evidence of microchimerism without prior pregnancy. To further understand this finding, a dam with prior male live births giving birth to her fourth litter of puppies, all females, was evaluated for FMC along with two of her daughters. All three female dogs had evidence of Y-chromosomal DNA in their blood. This suggests that male cells carried by the dam from previous pregnancy trafficked to her daughters to establish microchimerism in younger siblings. Companion dogs share many of the same cancers as humans, have out-bred genetics, and share the human environment, making them optimal models of human disease. Understanding the impact of FMC on health and disease of dogs could elucidate mechanisms useful for clinical interventions in humans.

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The health impact of fetal microchimerism (FMC) appears to range from contributions to disease causation to disease protection.[1-3] During the course of pregnancy, the feto-placento-maternal unit plays an important role in suppressing the maternal immune system, thereby facilitating the tolerance of fetal antigens.[4] The survival of fetal cells for years after pregnancy suggests persistence of this tolerance as well as the possibility that such cells could possess self-renewal and clonogenic properties reminiscent of stem cells. The presence of FMC in women has been linked to autoimmune and non-autoimmune diseases either in a protective or contributory role.[5] Studies have reported the contributory presence of FMC in autoimmune thyroid disease (Hashimoto's thyroiditis) and Graves disease, with a prevalence of 60% and 40%, respectively.[6,7] Existing data suggest a protective role for FMC in breast cancer, although the impact on clinical outcome is less studied and insufficient cases were analyzed to determine statistical significance for any given single parameter.[3] Interestingly, more recent work suggests that in breast cancer the correlation of FMC with cancer protection is nonlinear, thereby indicating that it can have both positive and negative consequences.[8] The persistent tolerized condition of FMC appears to reduce the likelihood of organ rejection and graft vs. host disease (GVHD) in transplant patients.[9,10] Elucidation of the true impact of FMC on these conditions and the therapeutic manipulation of FMC for patient benefit requires a relevant large-animal model of these diseases with naturally-occurring FMC. Humans' best friends, companion dogs, can provide just such a model.

The simplest way to identify the presence of FMC is to analyze the male microchimeric cells (due to unique presence of Y chromosome) in females with previous male pregnancy in their blood samples or isolated PBMC cells. We recently analyzed banked whole blood DNA samples from Golden Retriever dogs for FMC and identified Y-chromosomal material several years after parturition.[11] Companion dogs

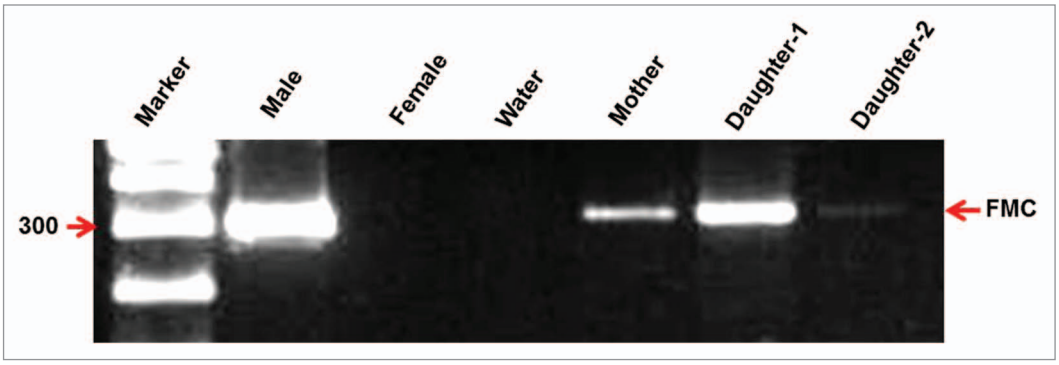

Figure 1. Gel electrophoresis of nested PCR assay for Y-chromosomal DNA in a dam and two daughters from her fourth litter of only female puppies. The first lane is the male control. The second lane is a negative female control. The third lane is a water control. An amount of 100 ng DNA was used as the starting template for the test subjects. The fourth lane is the dam which gave birth to three prior litters, two of which had male puppies. The fifth and sixth lanes are two daughters from a litter without male puppies.

have become a focus of interest in cancer research because of the outbred nature of these dogs, the similarities between human and canine cancers, and the shared environmental exposures of dogs and humans associated with carcinogenesis.[12] Companion dogs are frequently treated for osteosarcoma, lymphoma, mammary carcinoma, prostate cancer, and brain tumors, much like their human companions.[12-16] The identification of microchimerism in companion dogs now opens the door for both prospective and retrospective studies to determine association of FMC with disease risk and outcome. Y-chromosomal DNA was identified in each of the immediately post-parturient dogs reported previously, supporting the high rate of trafficking of fetal cells into the dam during pregnancy. In addition, the blood of nine nulliparous dogs contained Y-chromosome DNA. The source of the cells contributing the Y-chromosome is speculative, but sibling chimerism or maternal male chimeric cells trafficking into the fetus are most likely. To evaluate this further, the same PCR protocol as previously reported[11] was used to evaluate a dam and two puppies from an all-female litter for male microchimerism. The dam had three prior litters, the second and third of which contained male puppies. The delivery of her fourth litter was witnessed and she gave birth to four live puppies, all female. The results of PCR amplification of blood DNA of the dam and two puppies are presented in **Figure 1**. The dam is chimeric, presumably from her prior litters containing male puppies. Of the two female puppies, both were microchimeric with male cells. Amplification of the first puppy's blood DNA yielded a band of much greater intensity than either the dam's or the sibling's. The source of this microchimerism could potentially be from a resorbed male sibling that was never identified, but it is at least as likely that the male chimeric cells from the dam traversed the placenta and colonized the daughters. This is consistent with identified male chimerism in single female human babies.[17] The variation in intensity of the bands also raises interesting questions about the establishment of FMC. Were the puppies exposed to different doses of male cells in utero, is the maternofetal interface more or less permissive to cell translocation, or are there differential responses to cells crossing from the dam to foster greater or lesser chimerism? This interesting finding further strengthens the companion dog as a model to elucidate mechanisms of human FMC.

As transplantation medicine grows, relevant models for transplantation in diseased and treated individuals becomes increasingly necessary. The presence of FMC has been shown to lower the rates of rejection in human renal transplant recipients receiving HLA-haploidentical organs.[9] Mismatched hematopoietic stem cell transplants (HSCT) are more likely to engraft and less likely to cause serious GVHD in patients with FMC than in non-microchimeric patients.[10,18] Infusion of peripheral blood stem cells has resulted in regression of a thymic carcinoma from a mother to her microchimeric, haploidentical daughter without resulting GVHD.[10] Donor lymphocyte infusions are used therapeutically post-HSCT,[19] but may have application in microchimeric patients to generate anti-tumor immune responses. Recently, both auto- and allo-HSCT has begun to be clinically employed for the treatment of non-Hodgkin lymphoma in companion dogs.[20,21] These dogs offer an opportunity to evaluate the impact of FMC in the management of GVHD and in modifying conditioning regimens to decrease toxicity of the therapy while optimizing rates of engraftment and minimizing GVHD.

Further studies are ongoing to more fully characterize FMC in dogs. Our aim is to optimize detection of FMC to identify the presence and understand the function of these microchimeric cells in longitudinal studies. With the shorter lifespan and disease course of companion dogs, associations with disease and health can be more rapidly identified for application to human health.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Nassar D, Droitcourt C, Mathieu-d'Argent E, Kim MJ, Khosrotehrani K, Aractingi S. Fetal progenitor cells naturally transferred through pregnancy participate in inflammation and angiogenesis during wound healing. FASEB J 2012; 26:149-57; PMID:21974929; http://dx.doi.org/10.1096/fj.11-180695
2. Kallenbach LR, Johnson KL, Bianchi DW. Fetal cell microchimerism and cancer: a nexus of reproduction, immunology, and tumor biology. Cancer Res 2011; 71:8-12; PMID:21199793; http://dx.doi.org/10.1158/0008-5472.CAN-10-0618
3. Gadi VK, Malone KE, Guthrie KA, Porter PL, Nelson JL. Case-control study of fetal microchimerism and breast cancer. PLoS One 2008; 3:e1706; PMID:18320027; http://dx.doi.org/10.1371/journal.pone.0001706
4. Khosrotehrani K, Reyes RR, Johnson KL, Freeman RB, Salomon RN, Peter I, Stroh H, Guégan S, Bianchi DW. Fetal cells participate over time in the response to specific types of murine maternal hepatic injury. Hum Reprod 2007; 22:654-61; PMID:17074776; http://dx.doi.org/10.1093/humrep/del426
5. Fugazzola L, Cirello V, Beck-Peccoz P. Fetal microchimerism as an explanation of disease. Nat Rev Endocrinol 2011; 7:89-97; PMID:21178998; http://dx.doi.org/10.1038/nrendo.2010.216
6. Ando T, Imaizumi M, Graves PN, Unger P, Davies TF. Intrathyroidal fetal microchimerism in Graves' disease. J Clin Endocrinol Metab 2002; 87:3315-20; PMID:12107242; http://dx.doi.org/10.1210/jc.87.7.3315
7. Klintschar M, Immel UD, Kehlen A, Schwaiger P, Mustafa T, Mannweiler S, Regauer S, Kleiber M, Hoang-Vu C. Fetal microchimerism in Hashimoto's thyroiditis: a quantitative approach. Eur J Endocrinol 2006; 154:237-41; PMID:16452536; http://dx.doi.org/10.1530/eje.1.02080
8. Dhimolea E, Denes V, Lakk M, Al-Bazzaz S, Aziz-Zaman S, Pilichowska M, Geck P. High male chimerism in the female breast shows quantitative links with cancer. Int J Cancer 2013; 133:835-42; PMID:23390035; http://dx.doi.org/10.1002/ijc.28077
9. Joo SY, Song EY, Shin Y, Ha J, Kim SJ, Park MH. Beneficial effects of pretransplantation microchimerism on rejection-free survival in HLA-haploidentical family donor renal transplantation. Transplantation 2013; 95:1375-82; PMID:23519024; http://dx.doi.org/10.1097/TP.0b013e31828b10a1
10. Ichinohe T, Teshima T, Matsuoka K, Maruya E, Saji H. Fetal-maternal microchimerism: impact on hematopoietic stem cell transplantation. Curr Opin Immunol 2005; 17:546-52; PMID:16084712; http://dx.doi.org/10.1016/j.coi.2005.07.009
11. Axiak-Bechtel SM, Kumar SR, Hansen SA, Bryan JN. Y-chromosome DNA is present in the blood of female dogs suggesting the presence of fetal microchimerism. PLoS One 2013; 8:e68114; PMID:23861856; http://dx.doi.org/10.1371/journal.pone.0068114
12. Henry CJ, Bryan JN. Not lost in translation: how study of diseases in our pets can benefit them and us. Mo Med 2013; 110:216-9; PMID:23829106
13. Hansen K, Khanna C. Spontaneous and genetically engineered animal models; use in preclinical cancer drug development. Eur J Cancer 2004; 40:858-80; PMID:15120042; http://dx.doi.org/10.1016/j.ejca.2003.11.031
14. Bryan JN, Keeler MR, Henry CJ, Bryan ME, Hahn AW, Caldwell CW. A population study of neutering status as a risk factor for canine prostate cancer. Prostate 2007; 67:1174-81; PMID:17516571; http://dx.doi.org/10.1002/pros.20590
15. Axiak SM, Bigio A. Canine prostatic carcinoma. Compend Contin Educ Vet 2012; 34:E1-5; PMID:23532757
16. Villamil JA, Henry CJ, Hahn AW, Bryan JN, Tyler JW, Caldwell CW. Hormonal and sex impact on the epidemiology of canine lymphoma. J Cancer Epidemiol 2009; 2009:591753; PMID:20445802; http://dx.doi.org/10.1155/2009/591753
17. Dierselhuis MP, Goulmy E. We are all born as microchimera. Chimerism 2013; 4:18-9; PMID:23262442; http://dx.doi.org/10.4161/chim.23348
18. Dierselhuis MP, Blokland EC, Pool J, Schrama E, Scherjon SA, Goulmy E. Transmaternal cell flow leads to antigen-experienced cord blood. Blood 2012; 120:505-10; PMID:22627770; http://dx.doi.org/10.1182/blood-2012-02-410571
19. Chang YJ, Huang XJ. Donor lymphocyte infusions for relapse after allogeneic transplantation: when, if and for whom? Blood Rev 2013; 27:55-62; PMID:23261066; http://dx.doi.org/10.1016/j.blre.2012.11.002
20. Willcox JL, Pruitt A, Suter SE. Autologous peripheral blood hematopoietic cell transplantation in dogs with B-cell lymphoma. J Vet Intern Med 2012; 26:1155-63; PMID:22882500; http://dx.doi.org/10.1111/j.1939-1676.2012.00980.x
21. Lupu M, Sullivan EW, Westfall TE, Little MT, Weigler BJ, Moore PF, Stroup PA, Zellmer E, Kuhr C, Storb R. Use of multigeneration-family molecular dog leukocyte antigen typing to select a hematopoietic cell transplant donor for a dog with T-cell lymphoma. J Am Vet Med Assoc 2006; 228:728-32; PMID:16506937; http://dx.doi.org/10.2460/javma.228.5.728